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THE USE OF PHOSPHORUS OXYCHLORIDE IN THE SYNTHESIS OF AMINO ACID p-NITROANILIDES

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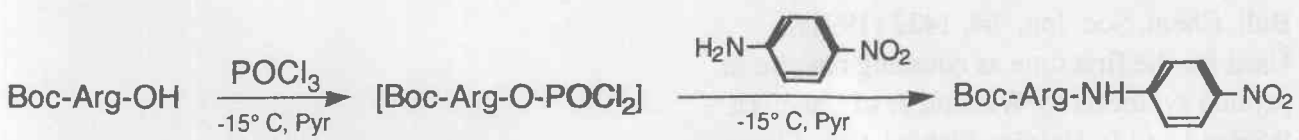
Abstract: Phosphorus oxychloride is described as a condensing agent in the preparation of nitroanilides. The condensation was free of racemization with amino acid derivatives of the urethane type and the current amino protective groups can be applied. The yields are high to excellent.

Amino acid p-nitroanilides are widely used as chromogenic substrates for the determination of the activity of proteolytic enzymes[1]. The synthesis of p-nitroanilides is rather difficult because of the low nucleophilicity of p-nitroaniline. The coupling methods used in peptide synthesis are for this reason inadequate[2]. The most frequently applied methods involve use of phosphorus trichloride (the phosphorazo-method)[3] or phosphorus pentoxide in diethyl phosphite[4]. With these methods the obtained yields vary between 30-44%. Of interest to us is arginine p-nitroanilide dihydrochloride, which can be used as a building block of e.g. chromogenic substrates. In the literature different syntheses of arginine p-nitroanilide are described[5]. These syntheses succeed only if protective groups were used, which are removable with strong acid.

Recently Noda *et al.* [6] described for the first time the synthesis of *tert*- butyloxycarbonyl amino acid p-nitroanilides via the pivaloyl mixed anhydride method. However, the synthesis of the arginine-derivative is not described. More recently Oyamada *et al.* [7] described the synthesis of *tert*- butyloxycarbonyl-arginine p-nitroanilide with the phosphorazo-method. This is the first time that the synthesis of a p-nitroanilide has been described in which the α-amino function is protected with a *tert*- butyloxycarbonyl group.

With the use of phosphorus oxychloride[8] in the synthesis of p-nitroanilides[9] nearly all protected amino acids can be used with virtually every α-amino protective group. We want to stress the usefulness of phosphorus oxychloride in the synthesis of N-protected arginine p-nitroanilide. We synthesized the hydrochlorides of Boc- (m.p. 187°C, α_D -12.8°, c=1 MeOH), Z- (m.p. 174°C, α_D -7.7°, c=1 DMF) and Fmoc- (m.p. 96°C (dec), α_D -37.2°, c=1 DMF) arginine p-nitroanilides in high yield (90-95%) and in an optical pure form[10]. In a typical procedure, (scheme) an α-protected amino acid (10 mmol) and p-nitroaniline[11] (10 mmol) were dissolved in dry pyridine[12] (30 ml). The clear yellowish solution was cooled to -15°C and phosphorus oxychloride (11 mmol) was added dropwise with vigorous stirring. During the addition the reaction mixture turned deep red and became turbid in the course of 15 min. The colour of the suspension slowly changed to orange, the reaction being complete after a total of 30 min (monitored by TLC). The reaction mixture was then quenched with crush-



Equimolar amounts of phosphorusoxytrichloride and carboxylic acid are required in this procedure.

ed ice and water (100 ml) and the nitroanilide was extracted into EtOAc. The combined EtOAc-layers were washed with saturated $NaHCO_3$ and NaCl solutions. The EtOAc-layer was not dried, to prevent premature crystallization and was directly evaporated *in vacuo* . The residue was coevaporated successively with toluene, EtOAc and MeOH to remove residual pyridine. To remove unreacted p-nitro-aniline, the crude reaction product was suspended in diethyl ether and filtered. The residue was subsequently recrystallized from a convenient solvent. With this method the following protected amino acid p-nitroanilides were synthesized in high yield (circa 90%) and in an optically pure form: Ala, Gly, Phe, Asp(OBu^t), Glu(OBu^t), Lys(Boc) and Arg(HCl).

We conclude that Wielands method[8] for carboxyl activation constitutes the method of choice for synthesis of amino acid p-nitroanilides.

References and notes:

1. B.J. Haverback, B. Dyce and H.A. Edmondson, Am. J. Med. **29**, 424 (1960); B.F. Erlanger, N. Kokowsky and W. Cohen, Arch. Biochem. Biophys. **95**, 271 (1961); H.C. Hemker ed. "Handbook of Synthetic Substrates for the Coagulation and Fibrinolytic System" Martinus Nijhoff Publishers, Boston, (1983).
2. With these methods only low yields are obtained after unnecessary long reaction times.
3. S. Goldschmidt and G. Rosculet, Chem. Ber. **93**, 2387 (1960).
4. G. Schramm and H. Wissmann, Chem. Ber. **91**, 1073 (1958); B.F. Erlanger and N. Kokowsky, J. Org. Chem. **26**, 2534 (1961).
5. E. Kasafirek, M. Chavko and M. Bartik, Coll. Czech. Chem. Commun. **36**, 4070 (1971); O. Somorin, N. Nishi and J. Noguchi, Bull. Chem. Soc. Jpn. **51**, 1255 (1978).
6. K. Noda, M Oda, M. Sato and N. Yoshida, Int. J. Pept. e Protein Res. **36**, 197 (1990).
7. H. Oyamada, T. Saito, S. Inaba and M. Ueki, Bull. Chem. Soc. Jpn. **64**, 1422 (1991).
8. Used for the first time as coupling reagent in peptide synthesis by Wieland *et al.* , see: Th. Wieland and B. Heinke, Liebigs Ann. Chem. **599**, 70 (1956).
9. The synthesis of an amino acid p-nitroanilide with phosphorus oxychloride was for the first time carried out by one of us (G.I.T.) and recommended by R.J. Planta and M. Gruber, Biochim. Biophys. Acta **89**, 503 (1964), who earlier obtained racemic Z-Gly-Phe-pNA in a low yield by activation of Z-Gly-Phe-OH with N, N'-dicyclohexylcarbodiimide, Anal. Biochem. **5**, 360 (1963).
10. The optical rotations found correspond with those cited in the literature, see 7. The behaviour of the p-nitroanilides synthesized by the method described here in enzymatic hydrolyses were catalogous to those obtained by other methods.
11. Recrystallized from toluene.
12. Pyridine was dried over KOH-pellets.